

## CLAIMS

*What is claimed is:*

1. A method of assessing the hepatotoxicity of a stimulus, the method comprising:
  - (a) exposing a hepatocyte culture to the stimulus;
  - (b) imaging the hepatocytes;
  - (c) analyzing an image of the hepatocytes to extract features characterizing the hepatocytes; and
  - (d) classifying the stimulus by quantitatively evaluating the extracted features to identify one or more hepatotoxic pathologies resulting from the stimulus, wherein hepatotoxic pathology classifications include two or more of the following: necrosis, cholestasis, steatosis, fibrosis, apoptosis, and cirrhosis.
2. The method of claim 1 wherein multiple cultures are located on a single support structure, and wherein each *in vitro* culture is exposed to a distinct stimulus.
3. The method of claim 2, wherein at least two of the cultures are exposed to different quantities of the same stimulus.
4. The method of claim 2, wherein the support structure is a glass or plastic support.
5. The method of claim 2, wherein the support structure is a multiwell plate.
6. The method of claim 2, wherein hepatocytes are co-cultured with support cells.
7. The method of claim 1, wherein the stimulus is exposure to a chemical compound.
8. The method of claim 1, wherein the hepatocytes are transformed or immortalized cells.

9. The method of claim 8, wherein the transformed or immortalized cells have been modified to express one or more cytochrome P450 metabolizing enzymes.

10. The method of claim 1, wherein analyzing the image comprises segmenting the image to identify individual hepatocytes on the image.

11. The method of claim 1, wherein the features extracted in (c) comprise two or more of membrane permeability, enzyme activity, Golgi distribution, migration of cytochrome c from the mitochondria, mitochondrial membrane potential, condensation, fragmentation and granularization of nuclei, accumulation of lipid containing vacuoles, bile production, actin morphology, and tight junction condition.

12. A method of identifying a necrotic hepatotoxic pathology resulting from a stimulus, the method comprising:

- (a) exposing a hepatocyte culture to the stimulus;
- (b) contacting the hepatocyte culture with markers for esterase activity and cell membrane permeability;
- (c) imaging the hepatocyte culture;
- (d) analyzing images of the hepatocyte culture to extract features relevant to necrosis;
- (e) identifying the average levels of esterase activity and cell membrane permeability for the hepatocyte culture based on the extracted features; and
- (f) characterizing the necrotic response of the hepatocyte culture to the stimulus based on the average levels of esterase activity and cell membrane permeability.

13. The method of claim 12, wherein the hepatocyte culture is characterized as necrotic if at least one of low esterase activity and high cell permeability is identified.

14. The method of claim 12, wherein the marker for esterase activity is calcein AM.

15. The method of claim 12, wherein the marker for cell membrane permeability is ethidium bromide homodimer.

16. A method of identifying an apoptotic hepatotoxic pathology resulting from a stimulus, the method comprising:

- (a) exposing a first and second hepatocyte culture to the stimulus;
- (b) performing a single step preparatory treatment of the first hepatocyte culture, wherein the single step preparatory treatment does not include washing the first hepatocyte culture;
- (c) performing a multi-step preparatory treatment of the second hepatocyte culture, wherein the multi-step preparatory treatment includes washing the first hepatocyte culture;
- (d) imaging the first and second hepatocyte cultures;
- (e) analyzing images of the first and second hepatocyte cultures to extract features relevant to apoptosis;
- (f) identifying condensation of the nuclei, cell adhesion, and average caspase-3 activity for the first and second hepatocyte cultures based on the extracted features; and
- (g) characterizing the apoptotic response of the first and second hepatocyte cultures to the stimulus based on the characteristics of the nuclei, cell adhesion, and average caspase-3 activity.

17. The method of claim 16, wherein the first and second hepatocyte cultures are characterized as apoptotic if at least one of condensation of the nuclei, lowering of cell adhesion, and increased caspase-3 activity are identified.

18. The method of claim 16, further comprising exposing at least one of the first and second hepatocyte cultures to a marker for DNA.

19. The method of claim 16, wherein cell adhesion is characterized by a washout coefficient.